GREATSOILS

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Use of organic and biological amendments in horticultural production systems and monitoring for any effects on soil and plant health

Raspberry

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This is one part of the final report of a 48 month project (Project 7 of the Soil Biology and Soil Health Partnership competed in 2021) which started in September 2017. The work was funded by AHDB and BBRO for £79,942.

CONTENTS

1. Abstract

As part of the work within the Soil Biology and Soil Health Research and Knowledge Exchange Partnership, this project explored the effects on soil and plant health of amendments in horticultural crop production; particularly their direct or indirect potential suppressive effects on soil-borne pathogens. In all the trials within Project 7 (onion, narcissus and raspberry), organic material was incorporated pre-planting to determine any benefits or otherwise to crop health. This trial examined raspberry cultivation in a commercial fruit farm field infested by V*erticillium dahliae*, the cause of Verticillium wilt.

A crop of a raspberry variety susceptible to Verticillium wilt was grown for three years in a field in which a Harris test had shown 41.6 viable *V. dahliae* microsclerotia/g of soil; 0.29 pg/g of *V. dahliae* were detected by qPCR. Six replicate plots (raised beds 0.8m wide) of three treatments, and an untreated control were set up in May 2018 on unsterilised soil. Anaerobic digestate solids (PAS 110 vegetable waste) were hand-applied at 50 t/ha (fresh weight) to 7m row lengths for two of the treatments and rotavated-in. Raspberry modules of a primocane fruiting variety were planted (14 per plot of 8 m), on 16 May 2018. On 21 May, 4 June and 22 October 2018 the biofungicide Prestop (*Gliocladium catenulatum*) was applied at 0.5% concentration to the planting holes of two of the four plots per replicate, one with and one without digestate. Treatment followed on similar dates in 2019 and May and June 2020.

No phytotoxicity occurred throughout the three years and there were no significant differences in crop vigour. In the unsterilised soil of the trial tunnel a mean 5% of stools wilted at the end of the first year; in the second year there was a transient wilt of 23% of the stools, but no significant treatment differences; but by the end of the third year, no canes were wilting. There were no significant treatment differences. As part of routine husbandry, old canes were cut out after fruiting and the strong new canes from the same stools were selected for fruiting. Fruit yield from the 24 plots at peak production on 11 July 2019 and 9 September 2020 did not differ between treatments.

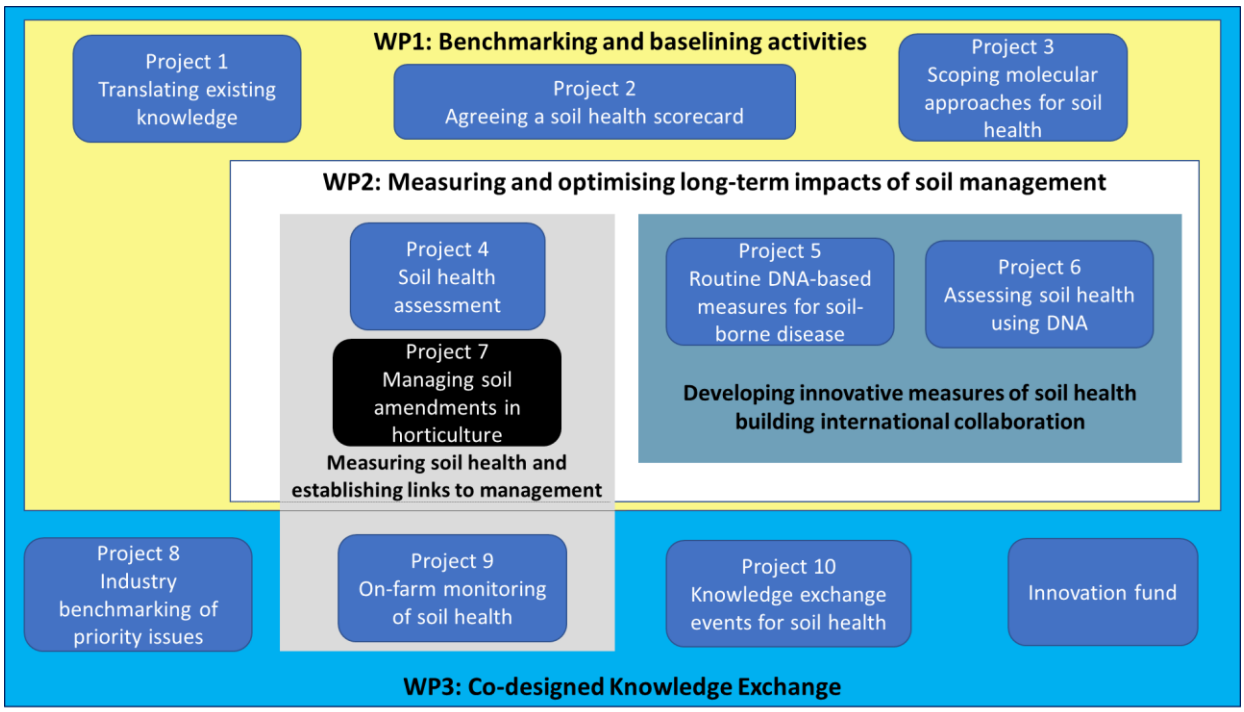
Overall, no benefit was shown over three years to crop vigour, fruit yield or expression of Verticillium wilt symptoms in raspberries from either a single incorporation of crop-based anaerobic digestate, or of up to three annual applications of a biofungicide. There were also no significant changes in soil health parameters developed over three years and the soil inoculum levels of *V. dahliae,* quantified at the end of the trial using the Harris test, was present at levels up to that seen before the addition of either digestate or biofungicide to the soil.

Quantitative PCR (qPCR) was successfully used to detect and quantify individual pathogens as well as biocontrol agents, including rhizosphere populations of *Gliocladium catenulatum*. However, quantification of *V. dahliae* in soil by qPCR requires further evaluation.

2. Introduction

This project is part of a suite of integrated projects within the Soil Biology and Soil Health Research and Knowledge Exchange Partnership (see Diagram below of how this project fits into the wider organisation of projects). This project (Project 7 of the Soil Biology and Soil Health Partnership – SBSH, together with Project 5) aimed to gain an understanding of any benefits gained from non-chemical inputs in horticultural cropping systems in the management of intractable soil diseases via potential changes to the soil microbial population and other biological, physical, and chemical aspects impacting on soil and crop health. This was focussed on a greater understanding of the effects on soil and plant health of amendments in horticultural crop production; particularly their direct or indirect potential suppressive effects on soil-borne pathogens.

Diagram to show how Project 7 (shown in black) fits within the integrated project delivery of the Soil Biology and Soil Health Research and Knowledge Exchange Partnership.



Inter-related objectives in Project 7 aimed to gain a better understanding of the soil biology and key soil health metrics that should be recorded by growers in order to be able to manage soils to be good for plant health and development:

1. To identify three fields with a history of fungal and/or oomycete soil-borne diseases and quantify the presence of up to six intractable soil pathogens by qPCR.
2. To carry out physical, chemical and biological assessments of the field soils in tandem with sampling for molecular assay and seek to determine any relationship between these.
3. To record changes in the soil microbiome following the use of soil amendments and determine any relationship between the microbial population composition and levels of disease in the crop.

Work on soil-borne diseases was carried out as part of the wider Project 07 within the Soil Biology Soil Health programme, each of the three crops studied (onion, *Narcissus* and raspberry) have been covered in separate reports (91140002-07a, -07b, and -07c). In all three crops, organic material was incorporated pre-planting to determine any benefits or otherwise to crop health. In raspberry, a plant protection product containing a beneficial fungus was also subsequently applied. The other two crops examined were narcissus (where a mycorrhizal product was applied at planting) and onion (where no products were applied).

Soilborne plant pathogens are among the most important limiting factors for UK horticultural crop production and build up with repeat cropping of susceptible hosts, often surviving between crops using resting spores. In raspberry, there are two diseases which can lead to plant death; Phytophthora root rot (with *Phytophthora rubi* usually being the dominant species) and Verticillium wilt. Soil disinfestation pre-cropping by chemical treatment has increasingly been restricted and the use of chloropicrin and dazomet products were no longer permitted in the UK from 2020. Varieties with resistance to *Phytophthora* spp. exist but are often not favoured by producer organisations and containerisation of the plants in peat or coir is often used to reduce, although not eliminate, the presence of the disease in the crop. Only Verticillium was detected in the site selected for the trial.

Verticillium albo-atrum and *Verticillium dahliae* (the latter species was split from *V. albo-atrum* in the 1970's) have a host range of over 300 woody and herbaceous plants. Microsclerotia, rather than just melanised tissue, are produced by *V. dahliae* allowing it to remain viable in soil for up to 14 years (Fradin & Thomma, 2006; Subbarao, 2020). Verticillium mycelium enters via roots and spreads through the xylem (Fradin & Thomma, 2006) and secretion of effector proteins that can suppress plant defence mechanisms has been reported (Lui et al, 2021). Different strains of *V. dahliae* may differ in pathogenicity. *V. dahliae* can be very destructive in raspberry and blackberry, resulting in stunted shoots, extensive wilting and ultimately plant death. Crop loss can occur if the canes die before reaching maturity, with severe outbreaks having occurred sporadically in UK cane fruit crops (Raffle and O'Neill, 2010). Some of the newer raspberry and blackberry varieties being planted by UK growers are derived from USA breeding lines with known high susceptibility to Verticillium wilt. Currently, growers sample soil before planting and send it for testing using the Harris test which involves growing the fungus from sieved-out microsclerotia (Harris *et al*., 1993). The results can take 6-7 weeks and so molecular techniques to quantify the pathogen in the soil in a fraction of that time utilising plantation sampling (Wedgwood *et al,* 2016) were sought to be refined as part of Project 5 within the SBSH Partnership, utilising soil samples from the present project. Quantitative real-time PCR (qPCR) provides a single platform for assessment of multiple target pathogens present in a single soil sample. There are many individual qPCR assays that have been developed for specific detection of soil-borne plant pathogens, including specific assays for *Verticillium dahliae* and related

pathogens such as *V. albo-atrum* and *V. longisporum* and a generic species-level qPCR assay for *Fusarium oxysporum*.

Some organic amendments such as composts and crop residues also have potential for controlling soilborne pathogens (Gamliel *et al*., 2000; Hoitink & Boehm, 1999; Noble & Coventry, 2005; Bonanomi *et al*., 2007; 2010; O'Neill, 2010). Increased soil organic matter content can cause beneficial changes to nutrient concentrations in plant tissues that make them more resilient to pathogen attack and can increase the microbial activity and effect changes in soil physico-chemical properties or structure, resulting in soils suppressive to specific pathogens. Anaerobic digestate solids were used on the raised raspberry beds of Project 7 rather than FYM or green compost (which were used in the onion and narcissus trials).

Soil features have been associated with disease suppression, but are variable, interacting and complex; they include physical, chemical and biological components. Biological plant protection products Serenade ASO (*Bacillus subtilis* strain QT 713) and Prestop (*Gliocladium catenulatum* strain J1446) are available to UK soft fruit growers as microbial soil / growing-media amendments. These act by competing with the pathogen in the rhizosphere, and have enzymatic (bacteria in Serenade ASO) or hyper-parasitic activity (fungus in Prestop) directly against the pathogen, but also stimulate the plants' own defence responses https://cropscience.bayer.co.uk/our-products/fungicides/serenade-aso/ https://icl-sf.com/uploads/UK/General_Downloads/Ornamental%20Horticulture/label_prestop_5_kg_uk_150x200_11-2020_v002.pdf . In the current project, Prestop was selected for testing, with molecular techniques to determine the organism's presence in the soil being investigated under Project 5.

3. Materials and methods

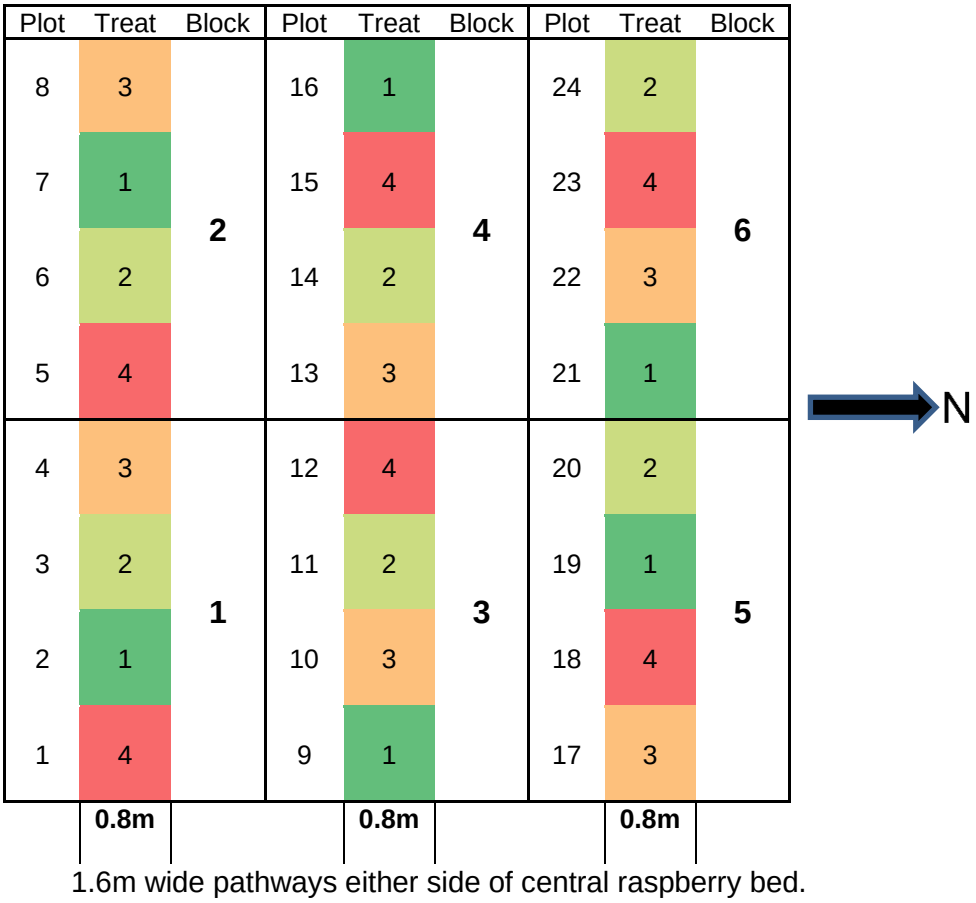
3.1. Treatment application and crop planting

3.1.1. Treatments and plot layout

In 2018, a four-year project was set-up in at Howes field in Norfolk (TG 327 204) where raspberries were to be grown between 2018 and 2020. The previous cropping was sugar beet (2011), spring barley (2012), winter barley (2013), sugar beet (2014), spring barley (2015), potatoes (2016) and spring barley (2017). The field was due to be planted commercially with raspberry modules of a primocane variety (name withheld) which is one of a number known to be particularly susceptible to both Verticillium wilt and Phytophthora root rot. The trial area was at the bottom of a field in which all the other beds were to receive chemical soil sterilisation by the grower following Harris test results showing a high density of *Verticillium dahliae* microsclerotia in the soil.

The trial area once set up contained three 0.8 m wide bed ridges with two 1.8 m wide middle pathways plus side paths to give a total width of 7.5 m. Each bed was divided into eight 8 m long plots, resulting in 24 plots. When all treatments were complete there were six replicate blocks of four randomised treatments (**Figure 1** and **Table 1**). The soil was covered with a plastic mulch with leaky hose irrigation underneath prior to planting. The planted plots were within a polythene tunnel which was covered each year by the grower during fruiting between May and October.

Two of the treatments received organic matter in the form of PAS 110 quality standard fibre digestate produced from anaerobic digestion of maize and vegetable wastes from a Cambridgeshire vegetable growing company. Separate samples of the digestate solids were taken from each of three layers of the bulk load as it was emptied onto the plots. The analysis of these layers gave consistent values and the means are given in **Table 3**. One of the treatments receiving the digestate subsequently also received biofungicide drenches of Prestop (*Gliocladium catenulatum*) to the raspberry plants, and a third treatment received only the biofungicide up to three times a year (**Table 2**). The fourth treatment was an untreated control and so received neither digestate nor biofungicide (**Table 1**).



1.6m wide pathways either side of central raspberry bed.

Central ten raspberry stools assessed per plot (two stools discard within ends of each 8 m plot).

Figure 1: Treatment randomisation with two replicate blocks per bed in trial tunnel in Howes Field.

Table 1: Treatments, rate and timing of applications to Howes Field raspberry bed ridges between May 2018 and June 2020.

Code	Treatment type	Rate	Application timing
T1	Untreated.	-	-
T2	PAS 110 anaerobic digestate (vegetable matter) solids.	50 t/ha fresh weight.	10 May 2018 onto beds & rotavated in before polythene covering & planting.
T3	Prestop drench (MAPP 17223).	0.5 g per 100 ml water applied per stool as 10% of root volume.	Two or three timings as detailed in **Table 2**.
T4	PAS 110 anaerobic digestate (vegetable matter) solids.	50 t/ha fresh weight.	10 May 2018 onto beds & rotavated in before polythene covering & planting.
	+		
	Prestop drench.	0.5 g per 100 ml water applied per stool as 10% of root volume.	Two or three timings as detailed in **Table 2**.

Table 2: Timing intervals between Prestop drench applications to T3 and T4 in 2018, 2019 & 2020.

Treat-ment code	**Application 1** Prestop in May at planting & repeated annually	**Application 2** In June, two weeks after first application	**Application 3** In October pre-senescence, but not needed in 2020
T1	Untreated	-	-
T2	Organic amendment.	-	-
T3	Prestop soil drench	Prestop soil drench.	Prestop soil drench.
T4	Organic amendment **+** Prestop soil drench	Prestop soil drench.	Prestop soil drench.

3.1.2. **Soil preparation and organic material application & analysis**

Soil preparation pre-treatment

When the soil was sampled in April 2018 it was found that ryegrass had been sown over 6 m width of the tunnel space to be used for the trial, with 1.5m still as weedy fallow. This was sprayed off with diquat herbicide by the grower by late April. Ploughing was then delayed by wet weather, but this was then carried out and three 0.8 m wide beds prepared by the grower in the area of the polytunnel on 9 May 2018.

Application of organic materials

On 10 May 2018, eight plots on each bed were marked out, each 8 m long, with 7 m of T2 and T4 plots to be treated, leaving 0.5 m as untreated guards at either end (thus 1m between neighbouring plots in a bed). The plots were marked out commencing 10 m in from where the beds started at the front of the tunnel space to give a discard of raspberry stools and finished at 64 m leaving 22 m of crop at the western end beyond the trial plot area.

Treatment calculations based on a rate of 50 t/ha used a treatment area of 1.6m width furrow to furrow i.e., the 0.8 m wide bed plus the adjacent half the width of the 1.6 m wide pathway between each bed, which over 7 m length equated to 11.2 m^2. This was based on the assumption that under commercial practice anaerobic digestate would be spread over the whole field surface before ridging-up, rather than what was to be done experimentally i.e., adding material to pre-formed beds so that not all plots would be treated. This resulted in a requirement of 55 kg of digestate solids per plot, which was weighed out in the field in buckets. The correct amounts were put onto sub-divisions of the length before spreading it out flat across the bed surface by hand. Fixed marker points were used to be able to relocate the plots.

The plots of T2 and T4 were covered by digestate, with two replicates per bed, and then a hand-guided rotavator was lifted onto the bed and the digestate incorporated into the top 150 mm (**Figure 2**). From the laboratory analysis of the digestate (**Table 3**) this resulted in 9.1 t/ha of dry matter which was estimated as giving an organic carbon loading of 2.72 t/ha (**Table 4**).

a) Digestate of shredded vegetable matter (pencil indicates particle size).

b) Digestate spread over 7m lengths of raised beds of two plots of T2 and T4 (looking east).

c) Digestate treatment following incorporation to 150 mm by rotavator.

Figure 2: Application of anaerobic digestate to half the plots at Howes Field on 10 May 2018. Showing a) Digestate texture b) Digestate spread on beds c) Digestate after incorporation into soil.

Analysis of organic materials

Table 3: Composition of the crop-based fibre digestate that was applied to Howes Field on 10 May 2018.

Dry matter (%)	N (kg/t FW)	P_2O_5 (kg/t FW)	K_2O (kg/t FW)	MgO (kg/t FW)	pH	NH_4N (kg/t FW)
18.1	4.6	4.3	6.4	2.0	8.8	0.4

Table 4: Total dry solids, nutrient and organic carbon loadings applied per ha to beds on Howes Field on 10 May 2018.

Dry matter applied (t/ha)	Organic carbon (t/ha)*	N (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Mg0 (kg/ha)
9.1	2.72	230	215	320	100

* Estimated loading assuming 30% Carbon for maize digestate (33.7% Carbon for food based)

RB209 guidance is that no more than 250 kg/ha Nitrogen should be applied.

3.1.3. **Raspberry planting & husbandry**

The three beds were re-profiled by the grower before laying irrigation hose down the lengths and covering with a black polythene mulch. Cuts were made in the polythene at about 0.5 m spacing down the bed length and coir-grown modules planted in a single line down the centre of each bed on 16 May 2018. This resulted in 14 plants within approximately every 8 m, with the central 10 plants per plot assessed for the trial.

To the north of the trial tunnel another 27 tunnels of the same variety were planted for the commercial crop within a few days of the trial tunnel crop. The soil in the commercial crop tunnels received chemical sterilisation before planting and a Paraat (dimethomorph) fungicide drench, but these treatments were withheld in the trial tunnel. All other crop husbandry (fertigation, herbicide, fungicide and insecticides) in the trial tunnel was carried out as needed as for the commercial crop. The grower also used hedgerow traps for the fruit fly *Drosophila suzukii* (Spotted Wing Drosophila / SWD).

Pruning was carried out by the grower as for the commercial crop. Canes that had fruited were cut out to allow new canes to grow and produce fruit. In 2018 the canes grown from the modules produced some fruit. These canes were then cut back after fruiting to leave two fully formed buds which then produced the cane that fruited in 2019. This can result in an uneven height of new cane growth depending on how short the floricane was cut. The 2019 fruiting canes were cut down with a hedge cutter after fruiting in order to desiccate any remaining fruit and thus reduce the build-up of

Spotted Wing Drosophila. The primocanes remaining were cut down at the start of 2020 and the new primocanes which grew in the Spring of 2020 then fruited in late Summer. When primocanes grew the first flush was killed off with contact herbicide and the second flush was thinned by hand to achieve the right cane density. The trial was discontinued in October 2020, but as the crop was still producing good yields the grower decided at that point to keep the field going with a floricane crop in 2021.

3.1.4. Bioprotectant product contents and application

Bioprotectant product application

Prestop (*Gliocladium catenulatum*, strain J1446, wettable powder $2x10^8$ cfu / g) was applied at the standard 0.5% concentration (5 g of product / L water) based on Authorisation 20181583 for protected edible crops. There was no product label recommendation on application volumes of drenching to soil-grown crops and so that of 10% root volume (frequently used for container-grown crops) was used. Therefore, for the first two drenches not long after planting of the module-grown plants, each rootball of one litre volume was given 100 ml of Prestop suspension, but this was doubled for all subsequent applications to the established plants as the root volume could only be estimated (**Table 1** and **Table 2**).

Tap water was used to make up the product. The suspension was, as recommended, left for 20 minutes after adding the product powder before topping up to the final volume and then stirred well before and during application. Application was carried out to the soil around the individual raspberry stools, pouring the liquid from either a measuring cylinder (for 100 ml) or a graduated beaker (for 200 ml) into the planting hole cut in the plastic mulch covering the bed (**Figure 3**). Applications were made to the 10 central plants of the 14 in each plot (leaving two discard plants at either end of the plot untreated) of T3 (Prestop) and T4 (anaerobic digestate solids, then Prestop). All six replicates were treated. The other two treatments were not given a water as a control drench at the same time but left untreated as they would be commercially.

In 2018, the first Prestop drench was done on 21 May once the modules had started to establish after planting on 16 May. A minimum interval of two weeks between applications was given on the product label and so the next application was given on 4 June 2018. A final, third, application was given on 22 October 2018 at the higher volume of 200 ml per stool before the leaves senesced and the plants stopped growing over winter.

In 2019, Prestop drenches of 200 ml per raspberry stool were given on 20 May, 5 June and 22 October. In 2020, the Prestop drenches of 200 ml per stool were only made on 22 May and 8 June, as the experiment was terminated in October.

21 May 2018

8 June 2020

Figure 3: Prestop drench to a newly planted raspberry plant in 2018, and in 2020 the last in a series of drenches at double the volume to well-developed raspberry stools. Howes Field.

3.2. Soil sampling

3.2.1. 2017 pre-crop soil sampling

On 14 November 2017, soil sample cores to 15 cm depth were taken across Howes Field in the cereal stubble using standard procedures (ADAS SOP SOILS/007). The sample was sent for both Harris testing to quantify the number of viable *V. dahliae* microsclerotia per gramme of soil using wet sieving of dry soil followed by culturing on agar (Harris *et al.,* 1993), and to determine the number of nematodes of different free-living species per litre of soil. Extractions were done after sieving soil through a 4mm sieve to remove stones and debris which would block the Seinhorst 2 flask transfer cone. These were carried out by the ADAS laboratory at High Mowthorpe.

A sub-sample of the soil was also sent for quantitative molecular testing (qPCR) using assay techniques (Bilodeau et al., 2012: Peters, 2012, and in-house protocols produced by James Woodhall) being further developed as part of the Soil Biology and Soil Health Partnership Project 5 within a PhD study based at the Fera laboratories near York. Work in AHDB project SF 097a showed that the Bilodeau method was able to detect down to 0.1 *V. dahliae* microsclerotia/g of soil and that there was a trend for the proportion of strawberry plants with Verticillium wilt to increase with increasing picogramme quantity of *V. dahliae* in their soil.

3.2.2. 2018 pre-crop soil sampling

On 19 April 2018, an area of 70 m long x 7.5 m wide was marked within the footprint of what was due to be the 48th tunnel in the field of commercially grown raspberries in Howes Field. No treatments had been applied. The area had not been due to be cropped and so there was a cover of seedling ryegrass, and also a 2 m wide strip of fallow along the south side of the tunnel, which were herbicide

treated with diquat later in April prior to crop planting. Each tunnel of 96 m length was due to hold three raised beds of a single row of raspberry modules. The trial area was marked out in the front of the tunnel (eastern end), the back quarter being due to hold a commercial crop. Allowing for 10 m of discard plants at the front of the tunnel and also some discard plants adjacent to the commercial crop at the far end, six replicate blocks were marked out; with each replicate being 32 m long and 2.5 m wide (i.e., including the location of future pathways).

A soil auger was used on 19 April 2018 to take 20 samples to 15 cm deep from within each of the six replicate blocks (**Figure 4** a & b). As standard procedure, topsoil samples were collected into a bucket and well mixed before sending 500 g samples for pH, extractable P, K and Mg, organic matter (loss on ignition and Dumas methodologies), total N, respiration (CO_2-burst), and to a separate laboratory able to give potentially mineralisable nitrogen (PMN). A 2 kg sample was also sent for qPCR. A 2 kg reference sample was returned to the Boxworth cold store. All samples were transported from the site in chilled cool boxes.

At the same time as the topsoil collection in April 2018, penetrometer resistance readings were taken to 30 cm depth at 20 locations within each of the six replicates (**Figure 4 c**). A soil pit 20 cm x 20 cm x 20 cm was dug in each of the replicates to assess for Visual Evaluation of Soil Structure (VESS) and count any earthworms according to standard procedures (**Figure 5**). On 22 October 2018, the soil around the central 10 plants in each of the 24 plots was sampled to 150 mm depth for qPCR before the Prestop drench was made.

a) Using a soil auger b) Topsoil sampled c) Penetrometer

Figure 4: Topsoil (to 150mm) sampling for nutrient analysis (a &b) and penetrometer testing (to 300 mm) (c) from each or six replicates marked out in ryegrass before herbicide treatment and ploughing. Howes Field 19 April 2018.

Figure 5: Visual evaluation of soil structure of soil on 19 April 2018 within six replicate blocks marked within the tunnel area on Howes Field due to be planted with raspberries in May 2018.

3.2.3. **2019 within-crop soil sampling**

On 5 June 2019, soil to 150 mm depth was collected for qPCR from the six replicates of the plots of T3 and T4 that had been treated 10 days before and were about to be re-drenched, and all the plots of the other two treatments. One soil core was taken from each of the ten plants in each of the 24 plots. On 26 November 2019 soil was again taken (for qPCR) with a 1.5 cm diameter corer from up to 150 mm depth of the 10 central planting holes of the 12 plots that had been treated with Prestop biofungicide on 22 October 2019. No soil sampling for nutrient analysis or visual assessment was carried out in 2019.

3.2.4. **2020 within crop soil sampling**

On 21 October 2020, the second sampling for topsoil nutrient analysis was carried out at the end of the experiment. Replicate Block 1, Block 3 and Block 5 only were sampled (which were in separate rows across the front part of the tunnel) with each of the four treatments sampled individually (to give 12 samples). Soil (2 kg) was also collected from these plots to send for Harris testing for viable *V. dahliae* microsclerotia and to quantify free-living nematode species.

The second set of penetrometer readings for the crop (three per plot) and visual assessments (one pit per plot) were carried out according to standard procedures on 21 October 2020 and necessitated partial removal of the polythene mulch over the raspberry beds in order to reach the soil. Soil samples were also taken on the same visit from each of the 24 plots for qPCR. Soil samples had been taken for qPCR from each plot at the time of the second Prestop drench on 5 June 2020, but the 50 g volume had been insufficient for multiple extractions.

The soil assessment results for the plots in 2018 and 2020 were each compared statistically by analysis of variance and compared using Duncan's multiple range test. Soil health scorecards were created based on treatment means.

3.1. Fruit harvest

Harvest records were not made for the first year of cropping in 2018 while plants became established. In 2019, fruit was weighed in the trial tunnel on one harvest date per plot, when fruiting was around its peak on 11 July. In 2020 the peak plot harvest was taken on 9 September in 2020. Marketable (Class 1) and unmarketable (Waste) berries were picked into separate punnets for each plot. Only the central 7 m of each plot was harvested from, across the positions of the central 10 stools, thus excluding branches spreading out sideways from the plots before and after in the row. Unmarketable fruit included those that were small or unripe as well as any with mould or distortion. Ten fruit per plot were also taken at random from the punnets of marketable fruit and weighed to obtain the average weight of a berry.

Fruit picking across the whole trial tunnel disregarding plot divisions was carried out by the farm staff every other day while in full production at the same intervals as the rest of the plantation. In 2019 fruit picking started on 22 June, peaking on 11 July, and finished on 1 August 2018. In 2020 because of the pruning timing used, the primocane was not destined to be producing fruit until late Summer and so picking commenced on 19 August, peaked on 12 September and had to finish early on 24 September 2020 following storm damage.

The total annual marketable fruit yield of the commercial tunnels was recorded by the grower and also that of the trial tunnel as a whole. Unfortunately, in 2020 the yield results from the trial tunnel were combined by the farm harvesting team with that of the adjacent tunnel.

3.2. Photographic and meteorological records

Photographs were taken throughout the trial to illustrate crop performance. Meteorological data was obtained for the period of the trial from "Irriguide METMAKER" and mean daily air temperature and rainfall calculated.

3.3. Crop vigour, wilting and phytotoxicity assessments

In Verticillium, the xylem in the stem becomes plugged by the pathogen, usually causing wilt to progress on leaves from the bottom to towards the top of the cane. Verticillium wilt can occur up one side of a stem and may produce a streaking visible up the stem. Conversely, Phytophthora can affect the leaves at the top of the canes before those lower down start to wilt, as the pathogen rots the roots and reduces their water uptake. Wilt symptoms from these pathogens can be expected to

develop faster in hot weather and would normally have a patchy distribution in the crop as the affected plants will be related to the distribution of soil inoculum.

Plant vigour, phytotoxicity and any yellowing or wilting was assessed at the time of each of the Prestop drenches in May, June and October, assessing the central 7 m of each plot. In 2020 it was not possible to distinguish which canes were growing from which stool because of the canopy density (with about 11 canes per stool), and so assessments were made at approximately 0.5 m intervals along each plot. The final assessment was made in October 2020 when soil samples were taken.

Where there was a range of vigour or symptoms an index was used to represent ranges of symptom severity, alongside a description of what was being scored, supported by photographs. Vigour records used a 0 to 9 index ranging from dead to excellent growth. Any phototoxicity would be recorded ranging from zero present to 9 (dead). For Verticillium wilt, an index used in previous research was utilised when seeking to record symptom severity as the disease progressed:

Index	Description of symptoms for Verticillium wilt
0	No symptoms
1	Pale wilting leaves
2	Weak looking plant pale wilted leaves
3	Cane discolouration & pale wilted leaves some leaf fall
4	Cane discolouration & all but the top leaves fallen
5	Death of canes

3.4. Molecular testing

Levels of *Verticillium dahliae* in the soil were monitored by qPCR detection. DNA was extracted from the soil using either the DNeasy PowerMax Soil Kit (Qiagen, Netherlands) as per manufacturer's instructions or an adapted version. Full method can be found in the report for SBSH Project 5. DNA was then stored at -20ºC until qPCR analysis. DNA samples were diluted 1:5 prior to qPCR analysis to minimise effects of inhibition. The assay used to detect *Verticillium dahliae* was designed by Bilodeau *et al*. (2012) and validated for use in soils by Kerr (2018). All qPCR reactions contained 1 x PCR Environmental Master Mix 2.0 (Applied Biosystems), primers and probes were added at concentrations of 7.5 µM and 5 µM (Eurofins, Integrated DNA Technologies) and 5 µl of extracted DNA was added in a total reaction volume of 25 µl, the remaining volume being made up with molecular grade water. qPCR cycling conditions were as follows: 10 minutes initial denaturation at 95 °C, followed by 40 cycles of 15 seconds denaturation at 95 °C and 1 minute primer extension at 60 °C. To quantify the amounts of *Verticillium dahliae* in the sample gBlock™ Gene Fragments (Integrated DNA technologies, US) were used in a serial dilution to compare against the sample results. Full method for this can be found in the Project 5 report (Elphinstone *et al.,* 2022).

4. Results

4.1. Crop vigour, wilting and phytotoxicity in the first crop year

Only a total of 5% of plants across the whole trial had wilted by the end of their first growing season (**Figure 6**) (at the time of the third Prestop application on 22 October 2018), too few to be able to demonstrate treatment effects (**Table 5**). Vigour was otherwise good, with a small production of fruit from the young plants taken by the farm but not recorded here, with the last fruit picked in October 2018.

No phytotoxicity was evident from any treatment in May, June or October 2018. The fruiting canes were cut down (as standard husbandry practice) in November 2018 to leave the primocanes that became the fruiting canes in 2019.

Table 5: Total number of raspberry stools (out of 60 stools i.e., 10 per plot and six replicates per treatment) showing any Verticillium wilting on 22 October 2018. Howes Field.

	Treatments					
	Un-treated	**Digestate solids**	**Prestop drench**	**Digestate + Prestop**	**Overall mean**	**Mean % of total**
Total wilting stools in crop	1	6	5	0	3	5

4.1. Crop vigour, wilting and phytotoxicity in the second crop year

By 20 May 2019 (at the first Prestop drench timing of the year), no wilt had developed on the canes that were growing in place of the previous year's fruiting canes. Although a few scattered plants looked a little stunted, vigour was generally good. By 5 June 2019 (at the second Prestop drench of the year) (**Figure 7**) there was no phytotoxicity, but wilting had developed across the tunnel, with a mean 14 in total per treatment, comprising 23% of the central ten plants/plot across the whole trial. There was no significant difference between treatments (**Table 6**). There was a wide range between replicate blocks in the proportion of stools wilting, coming close to a significant difference (P = 0.54) (**Table 7**) with a trend to fewer wilted in the central row (replicates 4 and 5) and in replicate 6 at the shadier northwest end of the tunnel than in rows of replicates 1 and 2 on the southern side and replicate 5 at the open eastern end. The number of stools with wilt per plot is tabulated in Appendix **Table 28**.

Figure 6: Two of the raspberry stools with wilting floricane towards the end of fruiting on 22 October 2018, both with symptomless primocanes also being produced from the same stool. Howes Field.

Figure 7: View eastwards in the tunnel from Replicate blocks 2 & 4 at the time of the second Prestop drench on 5 June 2019 when a few raspberry plants were showing wilt. Howes Field.

Table 6: Total number of raspberry stools (out of 60 per treatment) showing any wilting on 5 June 2019 and the mean % of plants affected per treatment. Howes Field.

	Treatments						15 df
	Un-treated	**Digestate solids**	**Prestop drench**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
Total wilting stools	12	16	18	10	14	-	-
% of stools wilting	20.0	26.7	30.0	16.7	23.3	18.62	0.432

Table 7: Mean % of raspberry stools in each replicate block showing any wilting on 5 June 2019. Howes Field.

	Replicate Blocks								15 df
	1	**2**	**3**	**4**	**5**	**6**	**Overall mean**	**L.s.d.**	**F value**
% of stools wilting	32.5	25.0	17.5	15.0	42.5	7.5	23.3	22.80	0.054

There was some variation in cane height on 5 June 2019 related to how the grower had cut down the canes that had fruited in 2018 in order to allow new canes to grow from well-developed buds and fruit in 2019. As cane die-back from disease might have resulted in small canes with bud break only half-way up the cane each of the ten plants per plot were recorded as small (less than 1 m), medium (1 to 1.5 m) and tall (over 1.5 m). Analysis was carried out distinguishing plants with canes either above or below 1 m, but there was no significant difference between treatments (**Table 8**).

Table 8: Mean % raspberry stools with canes above 1 metre tall on 5 June 2019. Howes Field.

	Treatments						15 df
	Un-treated	**Digestate solids**	**Prestop drench**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
% of plants over 1 m tall	65.0	65.0	48.3	66.7	61.2	41.52	0.759

When the peak fruiting harvest per plot was made a month later on 11 July 2019 wilting was only recorded in three stools (**Figure 8**) in Plots 1 and 12 (Digestate + Prestop) and Plot 4 (Prestop) with yellowing leaves and fruit production curtailed on these canes.

Figure 8: Dying leaves on two of the three stools in the fruiting crop wilting on 11 July 2019 following Verticillium infestation of the roots. Howes Field.

At the visit to apply the third Prestop drench on 23 October 2019, the floricanes had been cut back after the end of harvest and had been left hanging in the crop for the leaves to dry to aid in cane removal (**Figure 9**). It was therefore not possible to see if wilting had developed further in the floricanes. In contrast, in the 2018 and 2020 crop cycles fruiting continued into October. The primocanes were assessed instead in October 2019 and none were seen to have wilt symptoms.

Figure 9: Crop on 23 October 2019 with floricanes cut at the base and drying-off *in-situ*, with primocanes growing from the same stools showing no foliar wilting. Howes Field

4.2. Crop vigour wilting and phytotoxicity in the third crop year

When crop vigour was assessed at the second (final) Prestop drench of 2020 on 8 June, there was variation along the rows in crop height and so the plant canopy at each 0.5 m interval within the central row length of ten plants was assessed using a 0-9 vigour index based mainly on canopy height. The tallest canes (index 9) were at chest height (1.3 m), with index 7 at hip height (0.9 m) and index 6 were shorter and also tended to have sparser foliage (**Figure 10**). There was no significant difference between treatments, with a mean index of 8.3 indicating general good growth (**Table 9**) with little variation (**Table 10**). Neither wilting nor phytotoxicity was seen on 8 June 2020.

Table 9: Mean vigour index of raspberry stools (10 stools / plot). Howes Field, 8 June 2020.

	Treatments						177 df
	Un-treated	**Digestate solids**	**Prestop drench**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
Mean vigour index 0-9	8.233	8.367	8.317	8.333	8.312	0.2717	0.797

Table 10: Distribution of vigour index values for individual raspberry stools (10 per plot) showing the total number of stools of each index for each treatment. Howes Field, 8 June 2020.

	Vigour indices (0 shorter – 9 taller)									
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Untreated	0	0	0	0	0	0	1	6	31	22
Digestate	0	0	0	0	0	0	2	6	20	32
Prestop	0	0	0	0	0	0	0	8	25	27
Digestate + Prestop	0	0	0	0	0	0	1	6	25	28

Figure 10: View of tunnel from between Replicate Blocks 3 & 5 looking west, and a closer view of one plot to show variation in stool canopy height. 8 June 2020, Howes Field.

By 9 September 2020, at the time the plots were harvested individually, each plot's vigour overall was assessed, resulting in a mean index of 6.7 (moderate vigour) with the poorest index being 4 and no significant treatment differences (**Table 11** and **Table 12**). There was no wilting, only some leaf yellowing which may not have been caused by Verticillium, resulting in no significant treatment differences and a mean wilt index of 0.9, with indices no greater than index 2 (**Table 11** and **Table 13**). There was no significant difference between the replicate blocks in either wilt or vigour. Individual plot results are given in Appendix **Table 28**.

Table 11: Mean vigour and wilt severity indices on 9 September 2020 at peak fruit harvest.

	Treatments						15 df
	Untreated	**Digestate solids**	**Prestop drench**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
Mean vigour (0-9 index)	6.67	6.33	6.83	6.83	6.67	1.880	0.933
Mean wilt (0-5 index)	0.67	1.17	0.67	1.00	0.88	0.786	0.456

Table 12: Distribution of 0-9 vigour index scores between the six plots of each treatment in September 2020, showing vigour ranges making up the mean treatment vigour index.

	Vigour indices (0-9) per plot									
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Untreated	0	0	0	0	1	0	1	2	2	0
Digestate	0	0	0	0	2	1	0	0	2	1
Prestop	0	0	0	0	0	0	3	2	0	1
Digestate + Prestop	0	0	0	0	0	0	3	2	0	1

Table 13: Number of plots per treatment within each of the 0-5 wilt indices on 9 September 2020.

	Wilt index (0-5) per plot					
	0	**1**	**2**	**3**	**4**	**5**
Untreated	3	2	1	0	0	0
Digestate	1	3	2	0	0	0
Prestop	2	4	0	0	0	0
Digestate + Prestop	0	6	0	0	0	0

By 21 October 2020, when the final soil samples were taken, there were no wilt symptoms in the plants, but Autumn leaf senescence was occurring uniformly across the trial. Vigour assessed across each plot taking all stools together showed some variation between plots with an index range between 5 and 8, but the treatments did not differ statistically (**Table 14**). All except the combined treatments had some plots of index 5 or less, indicating poorer vigour (**Table 15**). There was no significant difference between vigour in the replicate blocks (data not shown).

Table 14: Mean 0-9 vigour index of raspberry stools on 21 October 2020 indicating moderate vigour across all treatments at the end of fruiting, Howes Field.

	Treatments						15 df
	Un-treated	**Digestate solids**	**Prestop drench**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
Mean vigour index 0-9	6.50	5.50	5.17	6.83	6.00	1.513	0.099

Table 15: The distribution of 0-9 vigour index scores between the six plots of each treatment on 21 October 2020 showing plots with poorer vigour except for in the Digestate + Prestop treatment.

	Vigour indices (0-9) per plot									
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Untreated	0	0	0	0	0	2	1	1	2	0
Digestate	0	0	0	0	1	2	2	1	0	0
Prestop	0	0	0	1	0	3	1	1	0	1
Digestate + Prestop	0	0	0	0	0	0	2	3	1	0

Relationship between crop vigour and microsclerotia counts at trial termination

Looking at the plot vigour results and comparing them with the microsclerotia counts from soil sampled from replicates 1, 3 & 5 on the same day in October 2020, there was no apparent relationship (such as poor vigour where there was more Verticillium inoculum in the soil), nor with the wilting that had temporarily been present in the plots in June 2020 (Appendix **Table 32**).

4.3. Fruit yields in second crop year

Fruit records were started in the second crop year after cane production had established.

Yield comparison between treatments

Fruit yield on 11 July 2019 did not differ significantly between treatments, with a mean 2.59 kg of Class 1 fruit and 0.38 kg of Waste fruit per 7 m of row (**Table 16**). The Waste, comprising a mean 13.05% of the day's harvest, was principally composed or overripe and unripe fruit (**Figure 11**) rather than being pest damaged or diseased. The Class 1 single berry weight was similar across the treatments, a mean 6.4 g (**Table 16**). There was no significant difference in the fruit records between replicate blocks (data not shown).

Table 16: Fruit harvest from the from the raspberry trial tunnel at peak fruit production from the central 10 stools within 7 m of row in each of the 24 plots on 11 July 2019. Howes Field.

	Treatments						15 df
	Un-treated	**Digestate solids**	**Prestop drench**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
Class 1 fruit yield (g)	2442	2553	2571	2796	2590	1030.5	0.903
Waste fruit (g)	366	345	514	331	389	452.6	0.812
Mean berry weight (g)	6.283	6.250	6.533	6.650	6.429	0.5833	0.417

Figure 11: Plot harvest at peak pick on 11 July 2019 showing Class 1 fruit (left picture) and a punnet of over and under-ripe fruit classed as Waste.

Comparison between overall trial tunnel and other farm crop harvests in 2019

Fruit was picked by the farm staff 19 times in the trial tunnel starting on 22 June 2019 with picking on alternate days between 2 July and the last pick on 1 August 2019 (full records not presented). Peak production was on 11 July 2019 and production started to peter out from the 24 July. In the whole tunnel harvest on 12 July, 82.86% was Class 1 thus 17.14% Waste (**Table 17**), whereas the mean for the individual plot records on 11 July 2019 was 86.94% thus 13.06% Waste (**Table 16**).

The Class 1 yield of the trial tunnel was 6821 kg/ha lower than the rest of the field by the final harvest (**Table 17**). Fewer fruit in total were picked in the trial tunnel, resulting in a total (Class 1 + Waste) yield of 15688 kg/ha compared with an average 22340 kg/ha from the other tunnels. By the end of harvest, the cumulative proportion of Class 1 fruit comprised 79.55% of the yield in the trial tunnel, so 20.45% Waste, compared with 86.40% Class 1 thus 13.60% Waste from the other tunnels (**Table 17**).

Table 17: Grower's fruit yield records from over the 2019 harvest period for the trial tunnel and other tunnels of the same variety in Howes Field. Weights of Class 1 fruit harvested and the calculated yields per hectare and proportion in Class 1.

	Total area harvested (ha)	Class 1 fruit (kg)	Waste fruit (kg)	Class 1 fruit (kg/ha)	Waste fruit (kg/ha)	% of total that was Class 1
Tunnel 48	0.072	898.57	231	12480.138	3208.333	79.55
Other 27 tunnels	2.332	45010.00	7086	19301.029	3038.593	86.40

4.4. Fruit yields in third crop year

Yield comparison between treatments

Fruit yield from the plots on 9 September 2020 did not differ significantly between treatments, with a mean 0.778 kg of Class 1 fruit and 0.210 kg of Waste fruit per 7 m of row (**Table 18**). The Waste comprised a mean 21.26% of the day's harvest, worse than the 13.06% in 2019. The Class 1 single berry weight was similar across the treatments, a mean 6.3 g (**Table 18**) similar to 2019. There was no significant difference in fruit records between replicate blocks.

Table 18: Fruit harvest at peak production. Class 1 and Waste yields and berry weight from the central 10 stools within 7 m of row in each of the 24 plots on 9 September 2020.

	Treatments						15 df
	Un-treated	**Digestate solids**	**Prestop drench**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
Class 1 fruit yield (g)	821	787	724	781	778	245.6	0.864
Waste fruit (g)	193	197	208	243	210	136.2	0.857
Mean berry weight (g)	6.228	6.250	6.573	6.300	6.338	0.5723	0.562

Comparison between overall trial tunnel and other farm crop harvests in 2020

Fruit was picked by the farm staff 19 times, every other day starting on 19 August 2020 in the trial tunnel and across the other 27 tunnels of the same variety in the field (full records not presented). On 9 September, production had entered its peak period with double the yield of the previous ten days, peaking on 12 September with tenfold the initial yields. Production was falling but still high when an unusually fierce storm ripped off a large proportion of the trial tunnel covering and also damaged other tunnels. Picking thus ceased on 24 September 2020.

It had been intended to record the yield of the whole trial tunnel separately to the other 27 tunnels of the same variety in the field, but a misunderstanding between the grower and the pickers meant they recorded the trial tunnel (tunnel 48) and the one next to it (tunnel 47) both together (both with three rows of 96m and 7.5 m wide). The trial plus neighbouring tunnel yielded 1892 kg/ha less Class 1 fruit over the whole harvest period than the other 26 tunnels and with 91.28% Class 1 thus 8.7% of the total yield was Waste (**Table 19**). However, the proportion of Waste varied from pick to pick and was 5.26% of the total on the 10 September 2019 the day after the plots were picked individually. At the individual plot pick, on 9 September 2019, in the trial tunnel four times that proportion of fruit (21.26%) was classed as Waste.

Table 19: Grower's fruit yield records from over the 2020 harvest period for the trial tunnel (Tunnel 48) plus adjacent Tunnel 47 and other tunnels of the same variety in Howes Field. Weights of Class 1 fruit harvested and the calculated yields per hectare and proportion in Class 1.

	Total area harvested (ha)	**Class 1 fruit (kg)**	**Waste fruit (kg)**	**Class 1 fruit (kg/ha)**	**Waste fruit (kg/ha)**	**% of total that was Class 1**
Tunnels 47 & 48	0.144	937	89.5	6506.944	621.528	91.28
Other 26 tunnels	2.256	18949	Not available	8399.379	n/a	n/a

4.5. *Verticillium dahliae* microsclerotia in soil samples

4.5.1. Microbial content pre-cropping

The Harris test on the soil taken from across Howes Field on 14 November 2017 following a spring barley crop indicated a total of 41.6 viable *V. dahliae* microsclerotia per gramme of soil.

Following qPCR of a sub-sample of the soil (also used for the Harris test) the DNA of *V. dahliae* was again detected, and in addition *V. longisporum* (a pathogen of oilseed rape) (**Table 20**). No *V. albo-atrum* was detected. No *P. rubi* was detected either, but this is a pathogen specific to cane-fruit and this crop had not been in the crop rotation since at least 2011. Overall bacterial and fungal content was quantified (**Table 20**).

Table 20: DNA content (expressed as picogrammes / gramme of soil) of pathogens and bacterial and fungal quantity in soil sampled across Howes Field after spring barley on 14 November 2017.

Verticillium longisporum **(pg/g)**	***Verticillium dahliae*** **(pg/g)**	***Verticillium albo-atrum*** **(pg/g)**	***Phytophthora rubi*** **(pg/g)**	**Bacteria U16S (Ct)**	**Fungi FQ (Ct)**
1.0	0.29	0.0	0.0	13.79	21.56

Ct (cycle threshold) the number of cycles required for the fluorescent signal to exceed background levels in real time PCR assays. The lower the Ct level the greater the amount of target nucleic acid in the sample. A positive reaction would have a Ct of 36 or less (Anon, undated).

4.5.2. Microsclerotia count comparison between treatments & replicates

There was no difference between treatments in the amount of viable *V. dahliae* microsclerotia present based on the samples taken on 21 October 2020 in replicates 1, 3 and 5, with a mean 21.6 per gramme of soil (**Table 21**). The amount in Block 1 (the row closest to the field bottom) ranked the highest with a mean 29.8 microsclerotia, resulting from three plots with higher-than-average microsclerotia counts (Appendix **Table 32**), but the difference was not significant statistically (P = 0.05) (**Table 22**). Replicate Block 1 included a 1.5 m wide strip along the tunnel wall that had been left as a weedy fallow when ryegrass as a headland had been sown in error by farm staff over the rest of the tunnel area prior to bed formation.

Table 21: Mean results for viable *V. dahliae* microsclerotia recovered using the Harris test from soil sampled in the raspberry plots on 21 October 2020 following Digestate incorporation pre-planting in May 2018 plus within-crop repeat Prestop drench applications to soil May 2018 to June 2020.

	Treatments						6 df
Viable *V. dahliae*	**Un-treated**	**Digestate**	**Prestop**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
Micro-sclerotia / g of soil	29.8	19.1	20.9	16.5	21.6	14.07	0.212

Table 22: Mean results for soil from three replicate blocks sampled in the raspberry plots on 21 October 2020 for viable *V. dahliae* microsclerotia (recovered using the Harris test). Howes Field.

	Replicates					6 df
Viable *V. dahliae*	**Block 1**	**Block 3**	**Block 5**	**Overall mean**	**L.s.d.**	**F value**
Micro-sclerotia / g of soil	29.8	19.1	20.9	21.6	12.19	0.050

Comparing the microsclerotia in the soil of the three replicate blocks (**Table 22**) with the incidence of wilting plants in June 2019 (**Table 7**) 17% of plants were wilting in Replicate 3, but the greatest proportion of wilted plants in all six replicates (42%) was in Replicate 5 whereas the mean microsclerotia density by October 2020 was similar in both replicated 3 and 5 so indicating no correlation between microsclerotia and the earlier recorded numbers of wilting plants (**Table 22**). Comparison of individual plot results confirmed the absence of any correlation between soil Verticillium levels and wilting attributed to *Verticillium* spp. (Appendix **Table 32**).

Looking at the individual treatment means it appeared there was a trend towards fewer viable microsclerotia being recovered in the three applied treatments, with the untreated ranking the highest density (**Table 21**). Therefore a 2 x 2 factorial analysis was done combining the values for the two digestate treatments and comparing these with the two Prestop treatments, plus without each treatment (**Table 23**), but no significant differences were shown because the range around the mean was too great (L.s.d. 9.95, 6 d.f.) with data from only the three replicates. There was no interaction between the Digestate and Prestop treatments (P = 0.468, 3 d.f.).

Table 23: Viable *V. dahliae* microsclerotia recovered by Harris test from soil sampled from around raspberries in Howes Field on 21 October 2020, comparing with and without each treatment.

	Microsclerotia counts per gramme of soil				6 df
Treatment	**Without treatment**	**With treatment**	**Overall mean**	**L.s.d.**	**F value**
Digestate	25.4	17.8	21.6	9.95	0.111
Prestop	24.4	18.7	21.6	9.95	0.207

4.5.3. **Molecular content of the soil during cropping**

Only, traces of *Verticillium dahliae* (mean = 0.2 ρg g^{-1} soil) were detected by qPCR in 2017 prior to planting the raspberry trial. No *Verticillium dahliae* was detected using the specific qPCR assay from the soil sampled subsequently in the crop during 2019 and 2020 as part of Project 5.

qPCR assays were optimised for quantification of rhizosphere soil populations of the biological control agent *Gliocladium catenulatum* using soil inoculation tests reported in Project 5.

4.6. Free-living nematode samples in soil

When Howes Field was sampled on 14 November 2017 before the raspberry crop there were:

1300 root lesion nematodes (*Pratylenchus* sp.) / L of soil (capable of damaging raspberry roots)
775 stunt / spiral nematodes (*Tylenchorynchus* sp.) / L soil.
25 stubby root (Trichodorus sp.) / L soil.
0 juvenile cyst nematodes (*Heterodera* sp.) / L soil.

When individual plots of raspberries were sampled in replicates 1, 3 and 5 on 21 October 2020 the only free-living nematodes then present were the root lesion and stunt species, with no significant difference between treatments (**Table 24**) or replicate blocks. Individual plot counts are given in Appendix **Table 31** and root lesion nematodes were principally below a yield loss threshold of 750 / L soil indicated by the ADAS sample laboratory. No significant differences arose from a 2 x 2 factorial analysis to compare with and without the two soil amendment materials (data not presented).

Table 24: Mean free-living nematode species recovered and counted per treatment following extraction from soil sampled 21 October 2020 after Digestate incorporation pre-planting in May 2018 and within-crop repeat Prestop drench applications to the crop between May 2018 and June 2020.

	Treatments						6 df
Nematodes / litre soil	**Un-treated**	**Digestate**	**Prestop**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**
Stunt	133	175	175	158	160	127.6	0.835
Root lesion	442	767	483	342	508	594	0.410

Soil sample results

4.6.1. Pre-trial soil sampling for soil health measures

The sampling of 20 soil cores per replicate block coincided with an unusually hot day for the time of year (19 April 2018), with soil temperature at 14°C at 10:00 h rising to 21°C by 15:00 h. The dryness of the soil increased the difficulty of collecting a full auger sample. Results for the six replicates for P, K and Mg are given in Appendix **Table 33** and VESS and penetrometer in Appendix **Table 34**.

4.6.2. Comparison of soil health before & after amendments & raspberry cropping

The topsoil analysis for 19 April 2018 for plots before giving any Digestate and planting the crop in May 2018 (Appendix **Table 33** and **Table 34**), and for 21 October 2020 at the end of the trial together with statistical comparisons are given in Appendix **Table 35**, **Table 36**, **Table 37**, **Table 38** & **Table 39**. Comparisons with background soil results, and any potential treatment effects are given below and summarised in soil health scorecards (**Table 25** and **Table 26**). In the scorecards the mean results are colour-coded according to the scorecard protocol; Red = Investigate, Amber = review, and Green = continue rotational monitoring. Results were assessed for the field being in a low rainfall region and light textured soil. Background information on the various soil health attributes and their measurement was given in an earlier SBSH Partnership report (Griffiths *et al.,* 2018).

Table 25: Soil health scorecard for pre-trial soil sampling of replicate blocks in Howes Field on 19 April 2018 before treatment incorporations.

Attribute	Site mean	Notes
pH	8.0	Potential for nutrient interaction
Ext P (mg/l) [Index]	50.6 [4]	see RB209 for guidance
Ext K (mg/l) [Index]	84.6 [1]	see RB209 for guidance
Ext Mg (mg/l) [Index]	49.8 [1]	Borderline index 1 see RB209 for guidance
SOM (% LOI)	2.2	
VESS score (limiting layer)	1.6	Friable – intact;
CO2-C (mg/kg)	71.2	Low activity
Earthworms (No./pit)	0	No worms - soil dry at sampling & hot weather

Table 26: Soil health scorecard for end of trial soil sampling in replicates 1, 3 and 5 of Howes Field raspberries on 21 October 2020. Notes on attributes shown below.

Attribute	Control	Digestate	Prestop	Digestate & Prestop
pH	7.7	7.5	7.5	7.5
Ext P (mg/l) [Index]	64 [4]	56 [4]	51 [4]	64 [4]
Ext K (mg/l) [Index]	172 [2]	160 [2]	125 [2]	176 [2]
Ext Mg (mg/l) [Index]	78 [2]	79 [2]	65 [2]	82 [2]
SOM (% LOI)	2.5	2.8	2.5	2.5
VESS score (limiting layer)	1	1	1	1
PMN (mg/kg)	31.2	25.8	31.8	23.2
CO2-C (mg/kg)	59.0	65.7	63.3	55.3
Earthworms (No./pit)	0	0	0	0

Soil type, pH and organic matter

The topsoil texture in Howes Field was a sandy silt loam with up to 12% clay. The pH after the raspberry crop and nutrient feeding given by the irrigation hose under the plastic mulch was unaffected by the treatments; pH was lower in 2020 compared to at the start of the experiment, and borderline for potential nutrient interactions (pH>7.5).. The soil organic matter (SOM%), in comparison with “typical” levels for the soil type and climate, was above average (> 2%) and there were no differences related to the treatments.

Earthworms & VESS

Before the raspberries were planted in 2018 no earthworms were found in the dry light soil, most likely compounded by the unusually hot dry sampling day. By the end of the trial in October 2020, sampling under the plastic mulch in the raised row ridges of the raspberry tunnel produced one earthworm from across the whole trial area.

There was no concern about the soil structure from the VESS pit samples in either the cereal stubble in 2018 or within the raspberry crop in 2020, with soils classed as having a friable or intact soil structure.

NPK

After three years of raspberry stool growth the level of extractable Phosphorus (P) had not changed following any of the treatments, being relatively high at Index 4, suggesting no external P inputs are required for the current raspberry crop.

The extractable Potassium (K) and Magnesium (Mg) status increased from Index 1 to Index 2, reflecting fertiliser inputs supplied via ”leaky hose” under the plastic mulch; there were no treatment differences.

Potentially mineralisable nitrogen (PMN) was not measured in the cereal stubble pre-trial, but in October 2020 at the end of observations, the plots that had received Digestate plus Prestop ranked lower (P = 0.05) than both untreated and Prestop only. The Digestate-only treatment ranked intermediately (**Table 27**). Both of the treatments with Digestate were recorded on the scorecard (**Table 26**) as below typical UK arable topsoil concentrations. It is unclear why levels were lower where digestate had been applied two years previously, however, there was a significant difference (P = 0.003) between the replicate blocks (**Table 27**), with the mean PMN value decreasing from Block 1 (mean 34.6) to Block 3 (28.6) to Block 5 (20.7), which suggests that underlying spatial variation may be responsible. There was little variation about each mean, with individual plot results of PMN in each replicate showed little overlap between the replicate blocks (Appendix **Table 36**).

Table 27: Potentially Mineralisable Nitrogen for individual raspberry plots to show how values decreased from Block 1 to 3 to 5 and that plots with both Digestate and Prestop (T4) tended to have lower PMN. Duncan's multiple range test. 21 October 2020.

	Treatments						6 df	Block
	Untreated	**Digestate**	**Prestop**	**Digestate + Prestop**	**Overall mean**	**L.s.d.**	**F value**	**F value**
PMN	31.16 b	25.80 ab	31.76 b	23.17 a	27.97	6.609	0.05	0.003

4.1. Weather data

Throughout the two years, conditions differed from the seasonal averages, with UK Meteorological office reviews reporting temperature peaks, extremes of rainfall and milder winters in 2019 and 2020 (https://www.metoffice.gov.uk/about-us/press-office/news/weather-and-climate/2019/weather-overview-2019 https://www.metoffice.gov.uk/about-us/press-office/news/weather-and-climate/facet/Year/2020).

The Irriguide measurements for daily mean temperature and rainfall are given in **Figure 12**. In April and May 2018 there were unusually hot Spring days while the plants were establishing. The 25 July 2019 was exceptionally hot and there were other days in June and September with sudden temperature peaks. In 2020 April, May, June and August also had days when temperatures were much higher than that of previous or following days. Temperatures would have been hotter under the polytunnel, increasing plant stress and hastening fruit ripening.

High rainfall occurred on 10 June and 6 October 2019 (over 30 mm), with over 40 mm on 24 September 2020 (**Figure 12**). However, the crop was under cover for most of the year. Irrigation by "leaky hose" ran along the beds under the black plastic mulch to ensure the crop received sufficient water during the growing season.

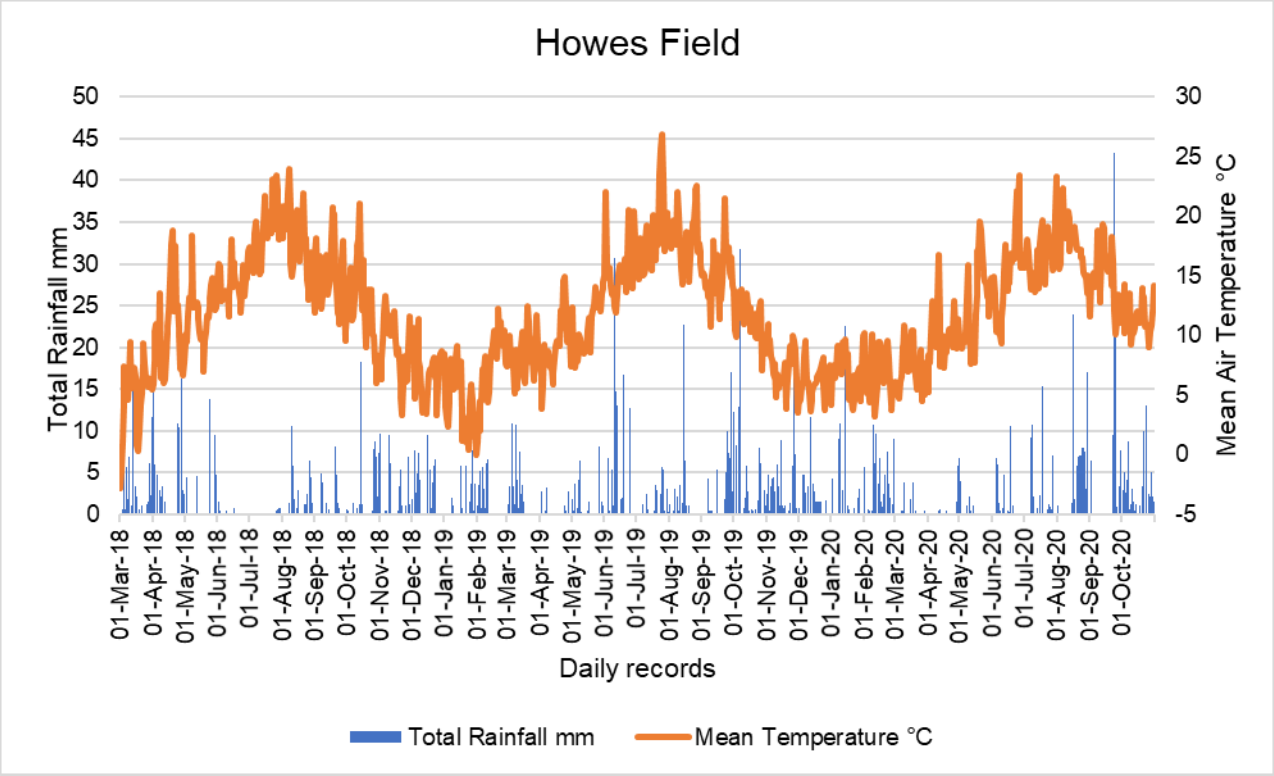


Figure 12: Total daily rainfall and mean daily air temperature for Howes Field near Cangate, Norfolk obtained from Irriguide METMAKER for the three years of raspberry cropping from March 2018 to 30 October 2020. Rainfall was less relevant to the crop when the tunnel was covered with polythene during flowering and fruiting from Spring to Autumn each year.

5. Discussion

5.1. The development of Verticillium in the crop

There is much ongoing work internationally on the management of Verticillium wilt, with a regularly updated review and extensive database of research papers maintained by CABI https://www.cabi.org/isc/datasheet/56275

There are no published thresholds for Verticillium microsclerotia in soil for raspberry, but in strawberry, two microsclerotia / g of soil can give complete crop loss (Cockerton *et al.,* 2019), with varieties differing in the density of propagules that they can tolerate. The raspberry variety used in the trial (not named for commercial sensitivity) is described as wilt sensitive, meaning that at counts of around ten microsclerotia per g of soil the plants could be expected to develop wilt or at least have reduced fruit production. However, fewer stools developed Verticillium wilt over the three years than had been expected by the high level of 41 viable microsclerotia/g of soil in November 2017, or the mean 21 viable microsclerotia/g of soil detected by another Harris test in October 2020. Other raspberry varieties such as Polka, Autumn Bliss and Autumn Treasure have been observed to not show symptoms of Verticillium infection even at more than 20 microsclerotia / g of soil provided they are growing in otherwise good soil conditions (Janet Allen ADAS soft fruit consultant, pers. comm.). It is possible that in the current project the cultivar was able to either not become infected, or more likely not show foliar Verticillium symptoms, because module plants were used that grew away strongly and never became drought stressed as a result of effective trickle irrigation. It may be possible that, when a wilt sensitive variety is only used for cropping over a 2-3 year period, it may be capable of tolerating and not succumbing to wilt even though the soil level of infection is high, if the plants establish well and only healthy cane is retained each year for further cropping. The pathogen could be unable to build up sufficiently to cause serious damage to such plants.

There was no relationship between the incidence of Verticillium seen in scattered plants in the crop June 2019 (when the maximum amount of wilt was recorded) and the *V. dahliae* microsclerotia numbers in the soil of those plots measured in October 2020. There are various reasons for this, one being that the wilting could have been related to some stools requiring more water than others, perhaps having grown more canes, but as water was supplied to all plants at the same rate, they were wilting physiologically in comparison with neighbours at the time of assessment. This could explain the apparent recovery of these stools at the time of further assessments. The Harris test only detects *V. dahliae*, not *Verticillium albo-atrum*, as the latter species does not produce microsclerotia and so cannot be sieved out of the soil and yet both *Verticillium* species can cause wilt. It is also not known whether *V. dahliae* microsclerotia that have germinated and infected plants then disintegrate or whether any viable cells of the microsclerotia are left and can be extracted by the Harris test. It is known that DNA in soils may be detected but not be from viable organisms. Where there are infected plants the microsclerotia around the roots may be “used up”, then only when the fungus has

exhausted the tissue it has infected does it produce new microsclerotia in the stems and roots. In the current project, no plant stools were killed. Any dying canes were cut out annually as part of the process of removing fruiting canes after harvest, and growers either remove these from the tunnel or chop them up in the pathways. In this growing system, the soil around the plants is covered by plastic sheeting and this means that the roots are shielded from any microsclerotia shed by any cut-out discarded diseased canes.

In a survey of plantations sampling raspberries and blackberries (Wedgwood *et al.*, 2016) fewer plants on sandy or silty loams showed Verticillium wilt (with *V. dahliae* confirmed in tissue using qPCR) than on clay loams and so this might have had a bearing on the lack of disease expression on the sandy loam of Howes Field. In the same project, *V. dahliae* was most successfully detected in stem base tissue, sometimes from roots and infrequently from soil.

The molecular techniques developed during Project 5 within this Soil Biology and Soil Health Partnership sought to improve the level of soil detection. Detection and quantification of pathogens was possible using purified pathogen DNA in the laboratory and qPCR assays were used to detect high inoculum concentrations in some soils freshly inoculated with most key soilborne pathogens (Project 5 report, Section 4.1.2). However, the methods used were not yet sufficiently sensitive to detect lower residual pathogen populations that would persist naturally in soils, especially for those pathogens with hardy resting spores from which it was difficult to extract DNA. It was therefore concluded that further optimisation of DNA extraction and purification methods would be needed before qPCR could be used for reliable quantification of plant pathogens across a range of naturally infested soils.

5.2. Verticillium levels in soil before and after treatment application

The preceding decade of cropping, including potatoes and sugar beet, as well as any dicotyledonous weeds with the cereal crops, would have allowed multiplication of *V. dahliae* in the soil resulting in the high initial level of 41 viable microsclerotia across the field. Hence the other tunnels on sites received chemical soil sterilisation. A patchy distribution of microsclerotia was shown in the plot sampling carried out at the end of the trial, with a wide range of 12 to 44 microsclerotia/g soil found within two metres of each other across the rows. A range of inoculum concentrations in a field complicates providing thresholds at which particular raspberry varieties might be grown, and not succumb to wilt, before the crop would normally be terminated. However, *V. dahliae* thresholds have been provided to UK growers to enable selection of strawberry varieties. For cotton plants, thresholds of 4 and 7 cfu/g soil from whole-field samples had potential to be set for susceptible and resistant cultivars, respectively (Wei *et al.,* 2015).

It was unfortunate that plots were not sampled individually at the start (as such a wide variation in levels was not expected), so it cannot be said whether there was actually a decrease in

microsclerotia density in 11 of the 12 plots sampled, but it is possible that microsclerotia were stimulated to germinate but then did not infect the plants and so lost viability.

Harris testing is quite labour intensive and costly with results taking twelve weeks. It was hoped that qPCR might be able to replace the Harris test, but as discussed in full in Project 5, further refinements of the extraction and detection methods are required for molecular quantification. Both yield and quality of extracted DNA is known to vary with the chemical and physical properties of different soils (Feinstein *et al.,* 2009) as well as the extraction method used (Petric *et al*., 2011). In addition, the PCR reaction can be inhibited to different extents by common soil components such as humic acids and phenolics and changes in magnesium and calcium levels (Wilson, 1997).

5.3. Effect of organic matter

No treatment differences in Verticillium propagule counts in the soil by October 2020 were seen after incorporating vegetable-based anaerobic digestate in May 2018. A similar lack of treatment difference was obtained in another AHDB project using digestate from the same source incorporated into *V. dahliae* strawberry beds, where the number of plants that subsequently wilted was used to indicate relative soil infestation levels (Xu et al., 2019). The addition of various organic soil amendments, both plant and animal derived, has been shown to be effective in reducing disease in some cropping situations (Conn et al., 1999; La Mondia et al., 1999; Lazarovits et al., 1999, 2000). This occurs primarily by affecting the survival of *V. dahliae* microsclerotia in soil and increasing populations of other components of the soil microflora. A limitation of this approach is that it is often only effective in certain soils, locations or cropping systems and may be totally ineffective elsewhere (Lazarovits et al., 2000).

In some irrigated crops, manipulation of soil moisture offers potential as a management technique for Verticillium wilt. In potato, early dying disease (caused by *V. dahliae*) is increased by excessive soil moisture during the first half of the growing season and therefore reducing early season irrigation may be a viable option to minimise disease losses (Cappaert et al., 1994). Reduced irrigation in spring via the seep-hose under the mulch would need to be investigated for raspberries, but it has been observed (Janet Allen, ADAS fruit advisor, pers. comm.) that it is when the water demand is at its greatest in summer during fruiting that symptoms of Verticillium wilting in the crop are more common.

The application rates of organic amendments are limited by regulations on annual nitrogen addition to the soil and, as soil-grown raspberry and strawberry crops are then covered by polythene to suppress weeds and retain soil moisture, no further applications are possible within the rows. With annual organic matter incorporation to fields prior to soft fruit cropping the water-holding capacity

could become increased so that after plant infection by Verticillium the parts of raspberry stools or strawberry crowns with roots still able to function could have a more-constant access to water and so survive longer.

Earthworm numbers were low, which is often the case in light textured soils, compounded by the hot sampling conditions at the start. Earthworms may have been present but may have burrowed deeper in the dry soil when it was assessed in April pre-cultivation. A high proportion of any nearer the surface at the time of bed-formation would have been killed during the production of the fine tilth. The loss rate with ploughing is about 25%, with rotating instruments up to 70% (Berner *et al.,* 2016). As the beds were polythene covered there would be minimal leaf debris available for any earthworms to feed on.

A review for CABI by Subbarao (2020) provides information that studies with nitrogen and phosphorus management have shown, in some cases, that providing optimal amounts of these nutrients can minimize Verticillium wilt (Pennypacker, 1989; Davis et al. 1994). The fertiliser source of nitrogen may also be significant (Elmer et al., 1994; Lazarovits et al., 2000).

Laboratory analysis of soil samples in both April 2018 pre-cropping and October 2020 gave respiration (CO_2-C) levels that indicated a low level of activity (a red / investigate rating on the soil health score card). Activity of the soil microbial biomass regulates organic matter transformations and associated energy and nutrient cycling. In general, an increase in respiration is considered beneficial and a decrease detrimental (Griffiths *et al.* 2018). Soil respiration is a measure of carbon dioxide (CO_2) released from the soil from decomposition of soil organic matter (SOM) by soil microbes and respiration from plant roots and soil fauna. It is an important indicator of soil health because it indicates the level of microbial activity, SOM content and its decomposition. The amount of soil respiration is an indicator of nutrient contained in organic matter being converted to forms available to crops (USDA undated). Soil organic matter was equally acceptable across both dates and the treatments. Further work is needed to determine whether the amount of fungal and bacterial DNA quantified in soil samples can be correlated with the microbial biomass carbon and activity levels.

5.4. Effect of beneficial fungus supplementation on plant growth

No differences in either crop vigour or wilting were seen whether Prestop was applied or not. This product is marketed as a protectant against soil-borne diseases (specifically listing species of *Pythium*, *Phytophthora*, *Fusarium* and *Rhizoctonia*) both out-competing pathogens for space on the roots and penetrating the pathogen's mycelium. The product application was made through the planting holes so it may be of minimal benefit to roots spreading out along under the polythene mulch. Up to three applications were made per year, but more could be used at a three-weekly

minimum interval, however the cost of this would need to be justified. Further research to provide guidance on the most appropriate utilisation of biofungicide drenches to protect against soil-borne pathogens is required, particularly if a period of beneficial root colonisation is needed before being challenged by the pathogen. Prestop applied to substrate grown raspberries in containers gave no significant reduction in root rotting by artificially inoculated *Phytophthora rubi* (Wedgwood *et al.,* 2020), nor in Viola to root rotting by *Thielaviopsis basicola* (Wedgwood, 2014). Another biofungicide permitted as a drench, Serenade ASO (*Bacillus subtilis*), resulted in significantly fewer wilted strawberry plants (15.5% v 37.9% in the untreated) when applied at planting into *V. dahliae* infested soil (Xu, *et al.,* 2019). With further research it is possible that molecular testing of the soil could be used to determine thresholds below which biological protection rather than chemical treatment would be effective and to link inoculum levels to likely disease severity in the crop.

qPCR assays were optimised for quantification of rhizosphere soil populations of *Gliocladium catenulatum* and further work will be needed to be able to utilise these molecular methods to assess the decline or multiplication of this beneficial fungus after Prestop application to soil or substrates.

5.5. Crop yield comparison between treatments and with the farm crop

Fruit yield was similar across the treatments, but this was to be expected because reduction would have followed the death of canes before fruit could be produced and ripen. The wilting seen on 5 June 2019 did not differ between treatments and did not progress (becoming absent in July) and also preceded the harvest period between 22 June and 1 August. In 2020, there was no wilt to reduce the yield. Verticillium wilt is known to become more apparent in soft fruit when the plants are stressed by fruit production in hot weather, but with the later harvest in 2020, water uptake would have been less of a problem.

The husbandry used in soil grown cane fruit crops destined to be kept for three or four years means that replacement canes are brought into production annually, so that provided the stool does not totally succumb to Verticillium then production can continue.

In 2019, less fruit/ha was produced from the trial tunnel than the same variety elsewhere in the field and there was a slightly reduced proportion of Class 1 fruit. In 2020, the trial tunnel and the neighbouring tunnel together also produced less fruit/ha than the rest of the tunnels, but the proportion of Class 1 had improved. It cannot be determined why less fruit was produced in the trial tunnel, but it is possible that withholding the soil from chemical sterilisation before planting allowed *Verticillium* spp. or some other soil organism/s to hold back the plants or that sterilisation stimulated some nutrient release as a result of biological turnover of the disrupted soil microbial populations. The position of the trial tunnel as the last in a line of 48 tunnels might also have had an influence on its production or the patterns of picking by farm staff.

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7. Appendix

Table 28 : The number of raspberry stools out of ten per plot that were wilting at five assessment dates of six replicates between 2018 and 2020. Old fruiting canes were cut out each Autumn as standard practice, and so records for each stool in the next year were for the newer canes present.

Plot	Treatment	Crop assessment dates of numbers of stools with wilt					0-9 wilt index per plot
		22.10.18	05.06.19	11.07.19	22.10.19	08.06.20	09.10.20
1	4	0	2	1	0	0	1
2	1	0	4	0	0	0	0
3	2	1	3	0	0	0	1
4	3	1	4	1	0	0	1
5	4	0	1	0	0	0	1
6	2	0	4	0	0	0	0
7	1	0	1	0	0	0	1
8	3	0	4	0	0	0	1
9	1	1	0	0	0	0	1
10	3	1	1	0	0	0	1
11	2	1	2	0	0	0	2
12	4	0	4	1	0	0	1
13	3	0	4	0	0	0	0
14	2	0	0	0	0	0	1
15	4	0	0	0	0	0	1
16	1	0	2	0	0	0	0
17	**3**	3	5	0	0	0	1
18	**4**	0	3	0	0	0	1
19	**1**	0	5	0	0	0	0
20	**2**	0	4	0	0	0	2
21	**1**	0	0	0	0	0	2
22	**3**	0	0	0	0	0	0
23	**4**	0	0	0	0	0	1
24	**2**	4	3	0	0	0	1
Total wilted		12	56	3	0	0	n/a

T1 Untreated, T2 Digestate, T3 Prestop & T4 Digestate plus Prestop.

In June 2019 out of 60 plants the number over six replicates wilting with potential *V. dahliae* was T1 12 stools, T2 16 stools, T3 18 stools and T4 10 stools.

Appendix

Table 29 : 11 July 2019 harvest of marketable fruit and 10-berry weight and unmarketable fruit from the area of 10 central stools per plot (7 m of row) at the peak of picking, Howes Field.

Plot	Treatment	Marketable fruit (g)	Mean weight of a marketable berry (g)	Un-marketable fruit (g)
1	4	2156	6.4	570
2	1	1933	6.3	120
3	2	2433	6.8	199
4	3	2421	6.7	101
5	4	2787	6.4	437
6	2	2331	6.5	379
7	1	2721	5.9	128
8	3	1908	6.7	236
9	1	2737	6.9	429
10	3	2684	6.3	658
11	2	2340	6.3	677
12	4	1296	6.9	439
13	3	1707	6.8	208
14	2	4320	5.2	285
15	4	3681	7.4	142
16	1	2270	64	687
17	3	2986	6.8	142
18	4	3175	6.8	0
19	1	3060	6.7	545
20	2	1394	6.3	299
21	1	1931	5.5	287
22	3	3718	5.9	1740
23	4	3678	6.0	397
24	2	2501	6.4	233

Appendix

Table 30 : 9 September 2020 harvest of marketable fruit and 10-berry weight and unmarketable fruit from the area of 10 central stools per plot (7 m of row) at the peak of picking. Vigour and wilting indices.

plot	Treat ment	Marketable fruit (g)	Mean weight of a marketable berry (g)	Unmarket-able fruit (g)	Vigour index (0-9)	Wilting index (0-5)	Phyto-toxicity index (0-9)
1	4	774.1	6.23	34.8	6	1	0
2	1	539.1	6.34	329.4	7	0	0
3	2	473.4	5.83	127.7	5	1	0
4	3	481.2	6.20	193.0	6	1	0
5	4	624.5	6.34	259.0	6	1	0
6	2	761.2	6.26	206.1	9	0	0
7	1	853	6.88	93.8	7	1	0
8	3	839.1	6.03	111.7	6	1	0
9	1	828.6	6.33	92.0	6	1	0
10	3	515.6	6.97	207.6	6	1	0
11	2	1192.5	7.07	107.5	4	2	0
12	4	664.5	6.78	224.6	6	1	0
13	3	783,8	7.05	296.5	9	0	0
14	2	896.3	5.72	109.8	8	1	0
15	4	1121.8	6.23	315.6	7	1	0
16	1	1129.3	6.16	117.4	8	0	0
17	3	1086.5	6.58	221.8	7	1	0
18	4	777.8	5.42	154.1	9	1	0
19	1	1021.9	6.25	140.8	8	0	0
20	2	592.4	6.04	381.2	4	2	0
21	1	553.4	5.41	382.4	4	2	0
22	3	637.6	6.61	218.1	7	0	0
23	4	723.0	6.80	468.8	7	1	0
24	2	809.1	6.58	248.4	8	1	0

Vigour and Wilt indices was based on that given in the methods – Vigour 9 excellent, Wilt index 2 had a little more wilting than the slight wilting of index 1. No phytotoxicity visible.

Appendix

Table 31 : Numbers of free-living nematodes(FLN) per litre of soil from samples sent to ADAS High Mowthorpe from within raspberry plots of replicates 1, 3 & 5 (at the tunnel eastern front) Howes Field on 21 October 2020 at the final visit to the crop.
Numbers of *V. dahliae* microsclerotia extracted per gramme of soil taken on the same date as the nematode samples. Both FLN and V.d. from the same 2 kg sample of soil)

			Numbers of nematodes of each species per litre of soil								
			Stubby Root	**Stunt/ spiral**	**Cyst juveniles**	**Root lesion**	**Needle**	**Dagger**	**Stem nematode**	**Root knot**	***Verticillium dahliae* propagules/g soil**
Plot	**Treat code**	**Treatments**	*Tricho-dorus*	*Tylencho-rynchus/ Helico-tylenchus*	*Hetero-dera*	*Praty-lenchus*	*Longi-dorus*	*Xiphi-nema*	*Ditylen-chus*	*Meloido-gyne*	
Plot 1	4	Digestate & Prestop	0	300	0	450	0	0	0	0	24.4
Plot 2	1	Untreated	0	175	0	475	0	0	0	0	44.8
Plot 3	2	Digestate	0	250	0	300	0	0	0	0	20.5
Plot 4	3	Prestop	0	175	0	825	0	0	0	0	30.4
Plot 9	1	Untreated	0	125	0	475	0	0	0	0	32.5
Plot 10	3	Prestop	0	125	0	400	0	0	0	0	15.1
Plot 11	2	Digestate	0	175	0	1100	0	0	0	0	21.9
Plot 12	4	Digestate & Prestop	0	75	0	300	0	0	0	0	12.3
Plot 17	3	Prestop	0	225	0	225	0	0	0	0	17.2
Plot 18	4	Digestate & Prestop	0	100	0	275	0	0	0	0	12.7
Plot 19	1	Untreated	0	100	0	375	0	0	0	0	12.1
Plot 20	2	Digestate	0	100	0	900	0	0	0	0	14.8

Appendix

Table 32 : Ranked Harris test results per plot of *V. dahliae* (V.d.) microsclerotia / g of soil collected from replicates 1, 3 and 5 within the raspberry tunnel in Howes Field in October 2020. Showing a trend to fewer microsclerotia in Row 3.

The number of wilting stools on 5 June 2019 out of 10 stools per plot (the date the greatest number of wilting stools was recorded) to show no rank correlation with microsclerotia counts in 2020.

Plot vigour index, where 0 = dead and 9 = excellent growth, on 21 October 2020 when no stools were wilting.

Row	Block	Plot	Treatment	Treatment code	*V. dahliae* / g soil 21.10.20	Number of wilting stools per plot 05.06.19	Plot Vigour index (0-9) 21.10.20
1	1	2	Untreated	1	44.8	4	7
2	3	9	Untreated	1	32.5	0	6
1	1	4	Prestop	3	30.4	4	5
1	1	1	Digestate & Prestop	4	24.4	2	7
2	3	11	Digestate	2	21.9	2	5
1	1	3	Digestate	2	20.5	3	6
3	5	17	Prestop	3	17.2	5	5
2	3	10	Prestop	3	15.1	1	5
3	5	20	Digestate	2	14.8	4	4
3	5	18	Digestate & Prestop	4	12.7	3	8
2	3	12	Digestate & Prestop	4	12.3	4	7
3	5	19	Untreated	1	12.1	5	8

Appendix

Table 33 : Topsoil analysis from six replicate blocks taken on 19 April 2018 before bed formation and incorporation of digestate into soil.

Replicate	Texture	% sand	% silt	% clay	pH	Ext P (mg/l)	Ext K (mg/l)	Ext Mg (mg/l)	SOM (%LOI)	CO_2-C (mg/kg) (CO_2 Burst)
Rep 1	Sandy Loam	53	37	10	8.1	52.4	99.3	53	2.3	60
Rep 2	Sandy Loam	52	38	10	7.9	49.2	86.8	52	2.3	78
Rep 3	Sandy Silt Loam	49	40	11	8.2	51.8	85	50.9	2.2	75
Rep 4	Sandy Loam	53	37	10	7.9	52	87.6	45.5	2.2	82
Rep 5	Sandy Loam	54	36	10	7.8	49.2	75.2	49	2.2	57.0
Rep 6	Sandy Silt Loam	44	45	11	7.8	49.4	73.7	48.4	2.2	75.0
MEAN	Sandy loam	50.8	38.8	10.3	8.0	50.7	84.6	49.8	2.2	71.2
Standard error		1.5	1.4	0.2	0.1	0.6	3.8	1.1	0.0	4.2

Appendix

Table 34 : Penetrometer records and visual assessment of soil in six replicate blocks on 19 April 2018 before raspberry bed formation.

Replicate	Bulk density	VESS score	VESS limiting layer	VSA score	Penetration resistance (Mpa)	Depth of resistance (cm)	Earthworms
Rep 1	1.524	1.0	1.0	27.0	0.89	22	0
Rep 2	1.699	1.4	2.0	25.5	0.95	16	0
Rep 3	1.797	1.5	1.5	27.0	0.89	15	0
Rep 4	1.689	1.5	1.5	27.0	1.27	29	0
Rep 5	1.642	1.3	2.0	27.0	1.06	14	0
Rep 6	1.52	1.2	1.5	27.0	1.03	9	0
MEAN	1.65	1.32	1.58	26.75	1.02	17.50	0.00
Standard error	0.04	0.08	0.15	0.25	0.06	2.86	0.00

Appendix

Table 35 : Topsoil analysis of samples taken within the raspberry plots of replicate blocks 1,3 & 5 on 21 October 2020. All plots sandy silt loam. For PMN see next table - statistical comparison between treatments and mean results are given in the subsequent two tables.

Plot	Code	Treat-ment	% sand	% silt	% clay	pH	Ext P (mg/l)	Ext K (mg/l)	Ext Mg (mg/l)	Ext. Na (mg/l)	Ext. Ca (mg/l)	SOM (%LOI)	Total N (%)	Ca CO3 (%)	SOC (%)	SOM (%) - calc	CO_2-C (mg/kg)
1	4	Digestate & Prestop	47	41	12	7.2	46.8	80	49.4	89.0	1541	2.4	0.099	<1	1.0	1.8	40
2	1	Untreated	49	40	11	7.6	62.6	170	81.9	64.4	1432	2.5	0.103	<1	1.1	1.9	50
3	2	Digestate	45	43	12	7.4	52.0	135	72.1	89.2	1599	2.5	0.107	<1	1.1	1.8	64
4	3	Prestop	49	39	12	7.3	52.2	135	72.4	124.0	1533	2.5	0.104	<1	1.1	1.8	69
9	1	Untreated	50	39	11	7.8	77.8	248	102.0	50.3	1222	2.5	0.106	<1	1.1	1.9	74
10	3	Prestop	45	43	12	7.6	54.6	126	62.3	98.6	1562	2.5	0.104	<1	1.0	1.8	64
11	2	Digestate	45	43	12	7.6	57.8	193	94.7	66.1	1304	3.3	0.135	<1	1.5	2.6	74
12	4	Digestate & Prestop	48	40	12	7.7	83.8	325	124.0	52.4	1406	2.6	0.113	<1	1.3	2.2	76
17	3	Prestop	49	39	12	7.6	45.4	114	58.9	106.0	1535	2.6	0.107	<1	1.2	2.1	57
18	4	Digestate & Prestop	47	40	13	7.5	61.0	124	73.6	75.0	1460	2.5	0.103	<1	1.0	1.8	50
19	1	Control	48	40	12	7.6	52.8	97	51.5	63.0	1512	2.4	0.115	<1	1.0	1.7	53
20	2	Digestate	44	42	14	7.4	59.2	153	70.0	56.8	1531	2.5	0.113	<1	1.0	1.8	59

Appendix

Table 36 : Potentially Mineralisable Nitrogen for individual raspberry plots to show how values decreased from Block 1 to 3 to 5 and that plots with both Digestate and Prestop (T4) tended to have lower PMN. Howes Field 21 October 2020.

Plot	Treatment Code	Treatment	PMN	Block Mean PMN
1	4	Digestate & Prestop	26.9	
2	1	Untreated	39.8	Block 1 34.6
3	2	Digestate	29.6	
4	3	Prestop	42.2	
9	1	Untreated	33.1	
10	3	Prestop	29.4	
11	2	Digestate	27.4	Block 3 28.6
12	4	Digestate & Prestop	24.7	
17	3	Prestop	23.7	
18	4	Digestate & Prestop	18.0	Block 5 20.7
19	1	Untreated	20.6	
20	2	Digestate	20.4	

Appendix

Table 37 : Topsoil analysis of final samples taken within the raspberry plots on 21 October 2020. Means of plots in Replicates 1, 3 and 5. Statistically significant differences - more Extractable sodium (Na) after Prestop (with Replicate 1 significantly higher than replicate 3) and block differences in % clay and pH.

Code	Treatment	Texture	% sand	% silt	% clay	pH	Ext P (mg/l)	Ext K (mg/l)	Ext Mg (mg/l)	Ext. Na (mg/l)	Ext. Ca (mg/l)
1	Control	Sandy silt loam	49	40	11	7.7	64.4	171.6	78.5	59.2	1389
2	Digestate	Sandy silt loam	45	43	13	7.5	56.3	160.3	78.9	70.7	1478
3	Prestop	Sandy silt loam	48	40	12	7.5	50.7	125.0	64.5	109.5	1543
4	Digestate & Prestop	Sandy silt loam	47	40	12	7.5	63.9	176.3	82.3	72.1	1469
	Mean from ANOVAR		47.17	40.75	12.08	7.525	58.8	158	76.1	77.9	1470
	F-value for treatments		0.057	0.122	0.059	0.135	0.316	0.737	0.702	0.002	0.293
	d.f.		6	6	6	6	6	6	6	6	6
	S.e.d.		1.217	1.089	0.385	0.0816	7.64	49.8	15.91	6.89	71.7
	L.s.d		2.978	2.664	0.942	0.1998	18.69	121.9	38.94	16.85	175.3
	Blocks F-value		n.sig.diff.	n.sig.diff.	0.037	0.016	n.sig.diff.	n.sig.diff.	n.sig.diff.	0.016	n.sig.diff.
					Rep 5 more clay	Rep 3 7.7 Rep 1 7.4				Rep 1 92 Rep 3 67	

Appendix

Table 38 : Topsoil analysis of final samples taken within the raspberry plots on 21 October 2020 continued. Means of plots in Replicates 1,3 and 5.

Code	Treatment	SOM (%LOI)	Total N (%)	CaCO3 (%)	SOC (%)	SOM (%) - calc	CO_2-C (mg/kg)
1	Control	2.5	0.11	<1	1.07	1.83	59.00
2	Digestate	2.8	0.12	<1	1.20	2.07	65.67
3	Prestop	2.5	0.11	<1	1.10	1.90	63.33
4	Digestate & Prestop	2.5	0.11	<1	1.10	1.93	55.33
Mean from ANOVAR		2.57	0.109	<1	1.12	1.93	60.80
	F-value	0.426	0.222		0.767	0.736	0.536
	d.f.	6	6		6	6	6
	S.e.d.	0.1851	0.0064		0.1312	0.2104	7.24
	L.s.d	0.4529	0.0156		0.3211	0.5148	17.72
	Blocks F-value	n.sig.diff	n.sig.diff		n.sig.diff	n.sig.diff	n.sig.diff

Appendix

Table 39 : Penetrometer results, VESS index & earthworm counts from within raspberry plots in replicate blocks 1, 3 & 5 on 21 October 2020.

Plot No.	Code	Treatment	Penetration resistance (Mpa)	Depth of resistance (cm)	VESS	Epigeic	Endogeic	Anecic	Juveniles	
1	**4**	Digestate & Prestop	1.53	26.7	1	0	0	0	0	
2	**1**	Untreated	1.40	26.7	1	0	0	0	0	Block 1
3	**2**	Digestate	1.33	30.0	1	0	0	0	0	
4	**3**	Prestop	1.33	30.0	1	0	0	0	0	
9	**1**	Untreated	1.12	28.3	1	0	0	0	0	
10	**3**	Prestop	1.05	30.0	1	0	0	0	0	
11	**2**	Digestate	1.35	28.3	1	0	0	0	0	Block 3
12	**4**	Digestate & Prestop	1.11	26.7	1	0	0	0	0	
17	**3**	Prestop	1.12	30.0	1	0	0	0	0	
18	**4**	Digestate & Prestop	0.76	30.0	1	0	0	0	0	Block 5
19	**1**	Untreated	0.63	28.3	1	0	0	0	0	
20	**2**	Digestate	0.81	31.3	1	0	0	0	0	
Means of Reps 1,3 & 5										
	1	Control	1.05	27.78	1					
	2	Digestate	1.16	29.89	1					
	3	Prestop	1.17	30.00	1					
	4	Digestate & Prestop	1.13	27.78	1					
Mean from ANOVAR			1.129	28.86						
		F-value	0.83	0.071						
		d.f.	6	6						
		S.e.d.	0.1423	0.888						
		L.s.d	0.3481	2.172						
		Blocks F-value	P=0.010							

Penetration	**Replicate Block**		
	1	**3**	**5**
Mpa	1.399	1.159	0.831